SUPPLEMENTAL INFORMATION

Figure S1

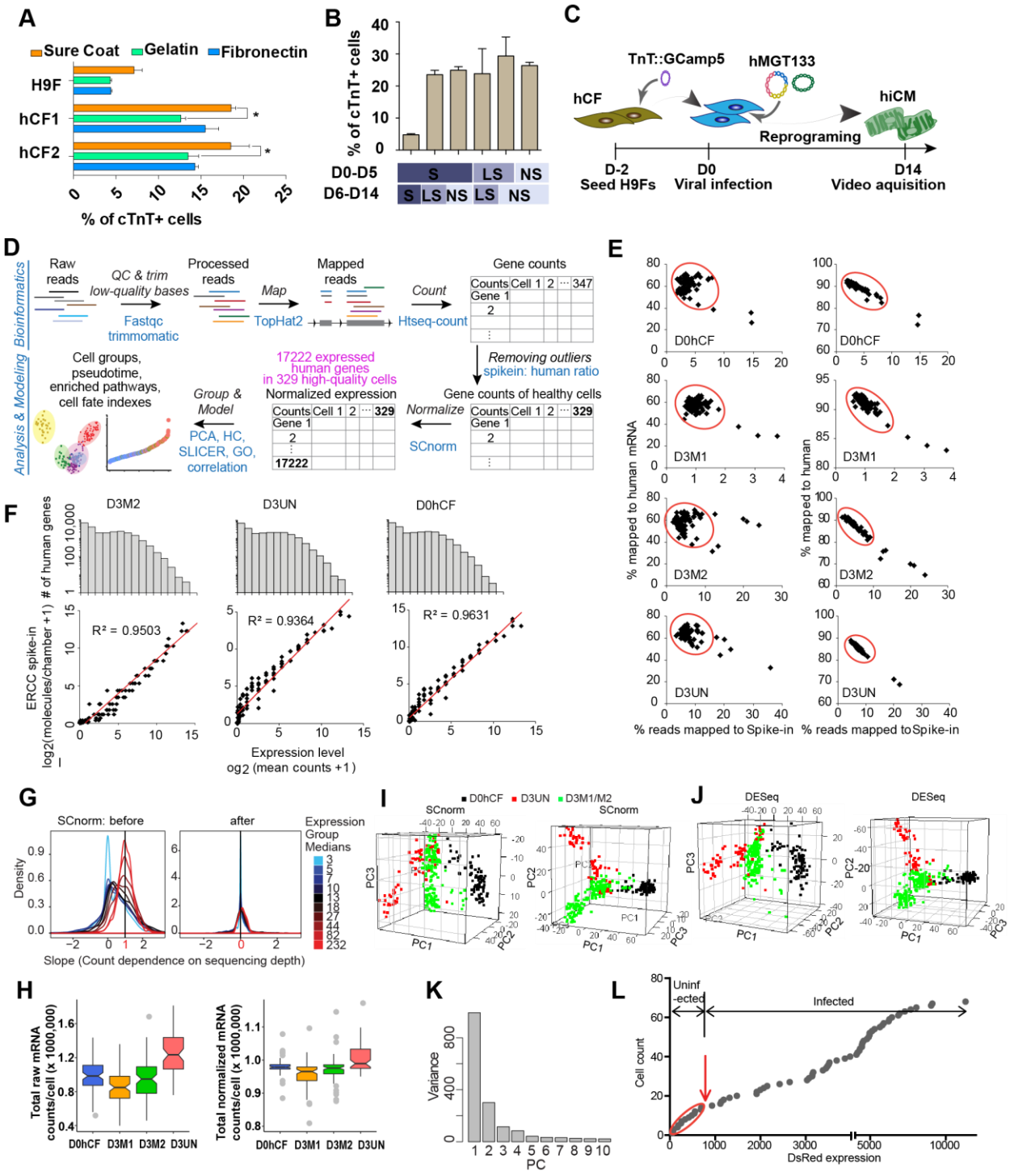


Figure S1. Related to Figures 1 and 2. hiCMs were generated from hCFs via infection of hMGT133. (**A**) Bar plot of FACS for cTnT+ cells 2 weeks after transduction of hMGT133 on

multiple types of hCFs cultured on different coating materials as indicated. (**B**) Quantification of flow analysis for cTnT+ cells on H9F cultured in normal serum (S), low serum (LS) OR no serum (NS) medium 14 days after induction of hMGT133. (**C**) Schematic of testing calcium oscillation in hiCMs by coinfection with a fluorescent calcium sensor in H9F. (**D**) Schematic of data analysis pipeline for scRNA-seq (see details in Methods). (**E**) Outlier removal. For each of the four single-cell experiments, the percentage of reads mapped to spike-in in each cell was plotted against percentage of reads mapped to human mRNA (left) or human genome (right) in that cell. Cells outside of the red circles are outliers. (**F**) Mean counts of each ERCC spike-ins were plotted against their theoretical molecule numbers in the lysis chamber (bottom) and the distribution of human gene expression level was plotted as histogram (top). Linear regression coefficient (R^2 value) is shown. Data from the D3M2, D3UN and D0hCF plates were shown as an example. (**G**) Density distribution of slopes, a measure of count dependence on sequencing depth, of different groups of genes expressed at various levels before (left) and after (right) SCnorm. (**H**) Box plots showing total raw (left panel) or normalized (by SCnorm, right panel) human mRNA counts per cell in each single-cell experiment. The central line indicates median. (**I, J**) Comparison of two normalization strategies, SCnorm (**I**) and DESeq (**J**), by 3D PCA score plots of all single-cell data. Two different angles were shown (left and right). Top 400 PCA genes in PC1, 2, and 3 were used. (**K**) PCA scree plot showing the variance of top 10 PCs. Related to Fig. 2G, H. (**L**) Normalized DsRed expression levels in each cell from the D3UN plate are shown. Because cells in this plate include 25% uninfected cells and 75% DsRed-infected cells from FACS sorting, the break point of the DsRed expression curve around 25% of cells was chosen as the cutoff: cells on the left of the breakpoint were classified as uninfected cells and cells on the right of the breakpoint were classified as DsRed-infected. Error bars indicate mean ± SEM, N=3. * $p < 0.05$ in (**A, B**).

Figure S2

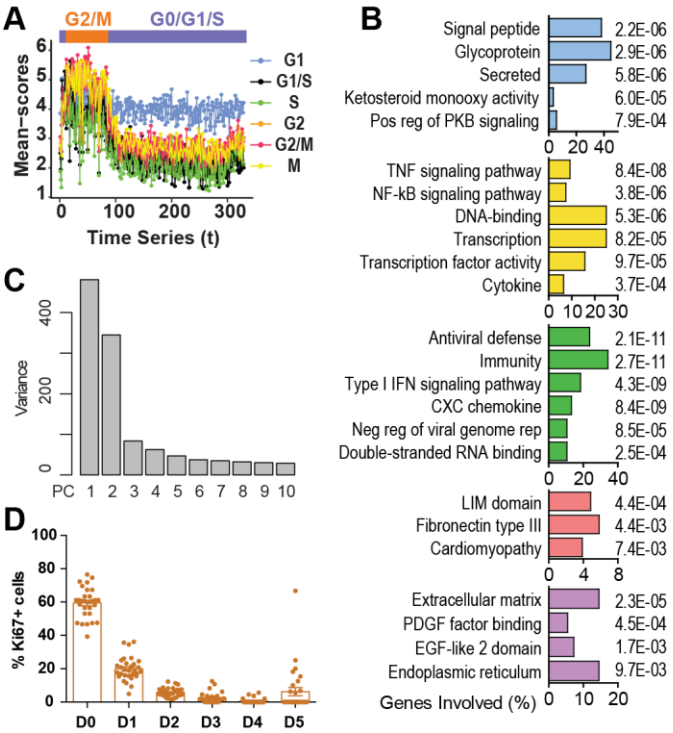


Figure S2. Related to Figure 2. scRNA-seq on day 3 reprogramming hCFs delineated cell cycle inhibition involved in reprogramming. (**A**) Each dot represents a cell and the cell cycle status of each cell was determined based on its Bayes score calculated by reCAT (Zehua Liu et al., 2017). The assignment of each cell's cell cycle phase was shown in a color bar on the right. **(B**) GO analysis with p values. Related to Fig. 3G. (**C**) PCA scree plot. Related to Fig. 3H, I. (**D**) Quantification of ICC for Ki67+ cells in hMGT133 infected hCF1 at different time point as indicated. Error bars indicate mean ± SEM, N=20 in (H).

Figure S3

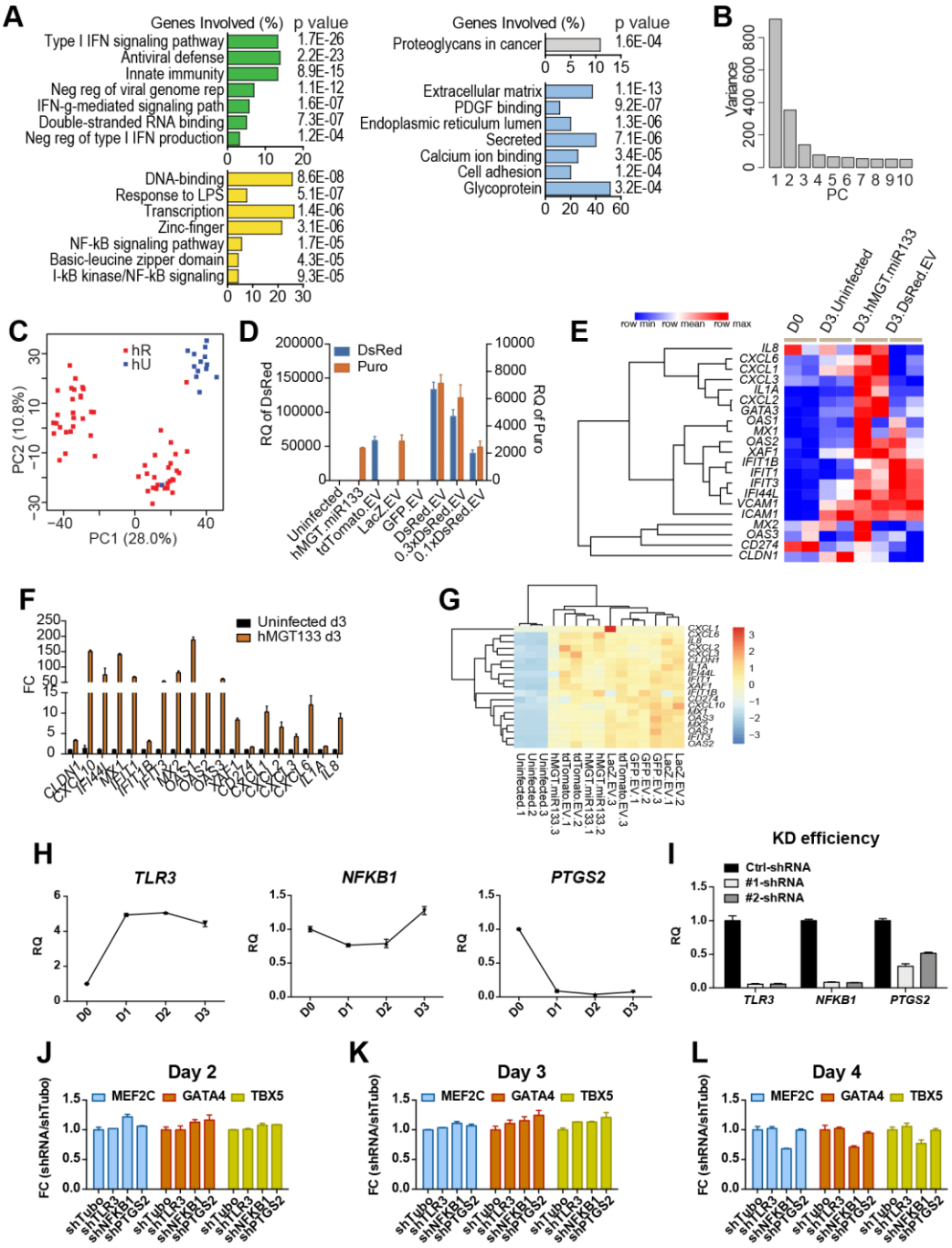
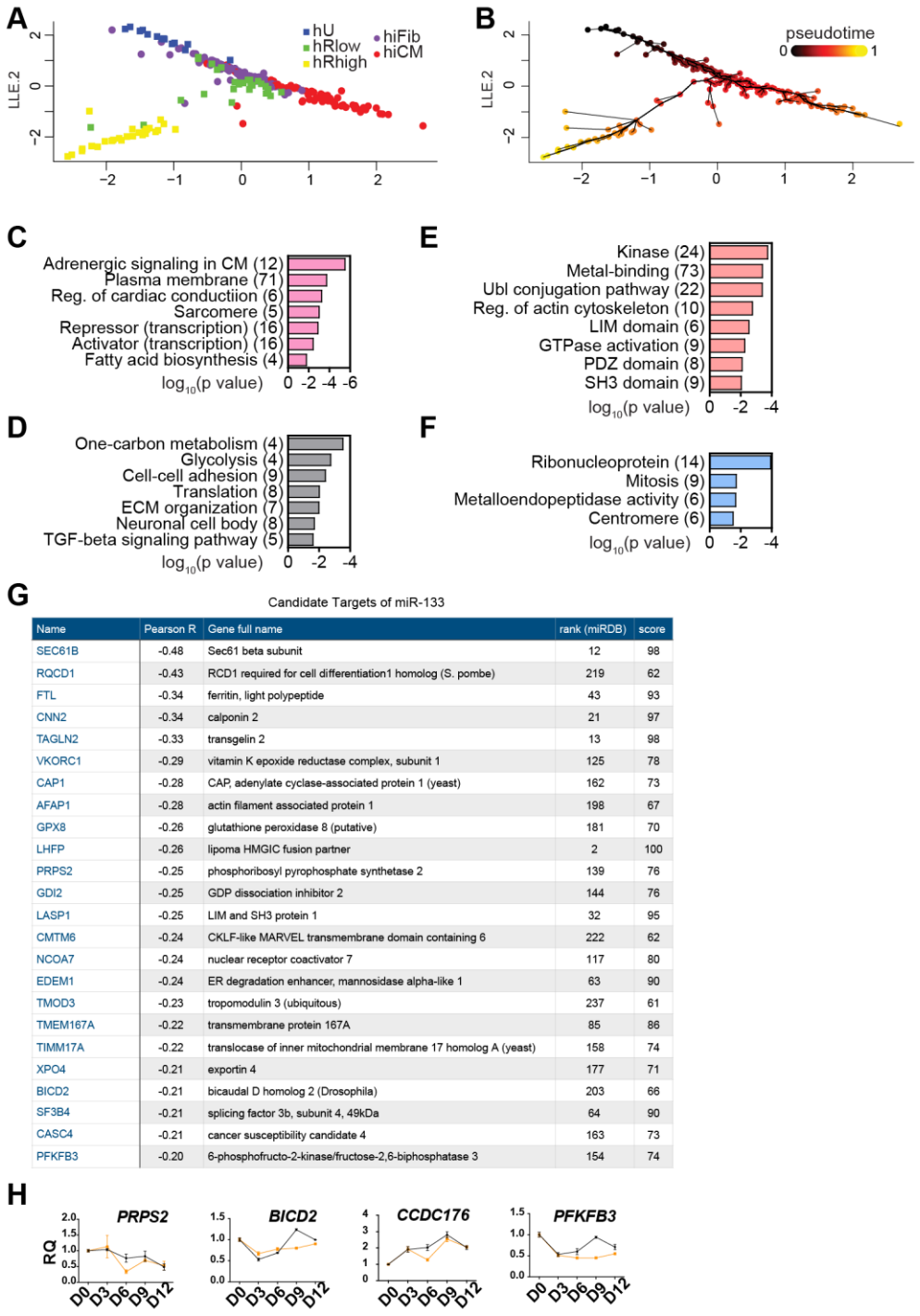


Figure S3. Related to Figure 3. The activation of immune response is potentially required for hiCM generation. (**A**) The same plot as Fig. 3A but with p values. (**B-C**) PCA scree plot (**B**) and score plot (**C**) of d3 control cells. Related to Fig. 3B, C. Cells in (**C**) were colored based on cell classification in Fig. S1L. This classification is different from HC in Fig. 3A and 3B by one cell. The HC cell groups were used in all following analysis. (**D**) Expression of DsRed and

Puromycin was determined by RT-qPCR in hCF1 on day 3 post infection of indicated virus. (**E**) Heatmap of the relative expression of indicated immune genes determined by RT-qPCR in uninfected H9F or H9Fs on day 3 post transduction of hMGT133 or LacZ-EV. (**F**) RT-qPCR analysis of indicated “antiviral response” genes at day 3 on hMGT133-infected and uninfected control hCF1. (**G**) Heatmap visualization of expression changes of genes associated genes in (**F**) in hCF1 uninfected or infected with indicated viruses. (**H**) Histogram showing the expression changes of *TLR3*, *NFKB1* and *PTGS2* determined by RT-qPCR at indicated time points. (**I**) Knockdown efficiency of each individual shRNA oligo measured by RT-qPCR. Expression values were normalized to those in EV-infected cells at day 14 post-transduction. (**J-L**) Normalized expression levels of indicated reprogramming factors determined by RT-qPCR after transduction of indicated shRNA lentiviruses at day 2 (**J**), day 3 (**K**) and day 4 (**L**). Expression value of each gene was normalized to that in shNT-infected cells. Error bars indicate mean ± SEM, N=3 in (**D**, **F** and **H-L**).

Figure S4



Name	Pearson R	Gene full name	rank (miRDB)	score
SEC61B	-0.48	Sec61 beta subunit	12	98
RQCD1	-0.43	RCD1 required for cell differentiation1 homolog (S. pombe)	219	62
FTL	-0.34	ferritin, light polypeptide	43	93
CNN2	-0.34	calponin 2	21	97
TAGLN2	-0.33	transgelin 2	13	98
VKORC1	-0.29	vitamin K epoxide reductase complex, subunit 1	125	78
CAP1	-0.28	CAP, adenylate cyclase-associated protein 1 (yeast)	162	73
AFAP1	-0.28	actin filament associated protein 1	198	67
GPX8	-0.26	glutathione peroxidase 8 (putative)	181	70
LHFP	-0.26	lipoma HMGIC fusion partner	2	100
PRPS2	-0.25	phosphoribosyl pyrophosphate synthetase 2	139	76
GDI2	-0.25	GDP dissociation inhibitor 2	144	76
LASP1	-0.25	LIM and SH3 protein 1	32	95
CMTM6	-0.24	CKLF-like MARVEL transmembrane domain containing 6	222	62
NCOA7	-0.24	nuclear receptor coactivator 7	117	80
EDEM1	-0.24	ER degradation enhancer, mannosidase alpha-like 1	63	90
TMOD3	-0.23	tropomodulin 3 (ubiquitous)	237	61
TMEM167A	-0.22	transmembrane protein 167A	85	86
TIMM17A	-0.22	translocase of inner mitochondrial membrane 17 homolog A (yeast)	158	74
XPO4	-0.21	exportin 4	177	71
BICD2	-0.21	bicaudal D homolog 2 (Drosophila)	203	66
SF3B4	-0.21	splicing factor 3b, subunit 4, 49kDa	64	90
CASC4	-0.21	cancer susceptibility candidate 4	163	73
PFKFB3	-0.20	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	154	74

Figure S4. Related to Figures 4. RNA velocity analysis of cell movement along the trajectory and downstream gene networks regulated by reprogramming factors. (**A-B**) Reprogramming trajectory constructed by SLICER showing HC/PCA cell groups (**A**) or

pseudotime (**B**). LLE, local linear embedding. (**C-F**) GO analysis showing p values. Related to Fig. 4C (**C** and **D**) and Fig. 4E (**E** and **F**). (**G**) List of miR-133 candidate targets selected based on the miRDB prediction (Score >= 60) and Pearson's correlation coefficient (R <= -0.2) between miR-133 level and the target gene in each single cell with high expression level of MEF2C (expression value > 200). (**H**) Relative expression of indicated candidate targets of miR-133 in hMGT- or hMGT133-infected cells along the time course. Error bars indicate mean ± SEM, N=3.

Figure S5

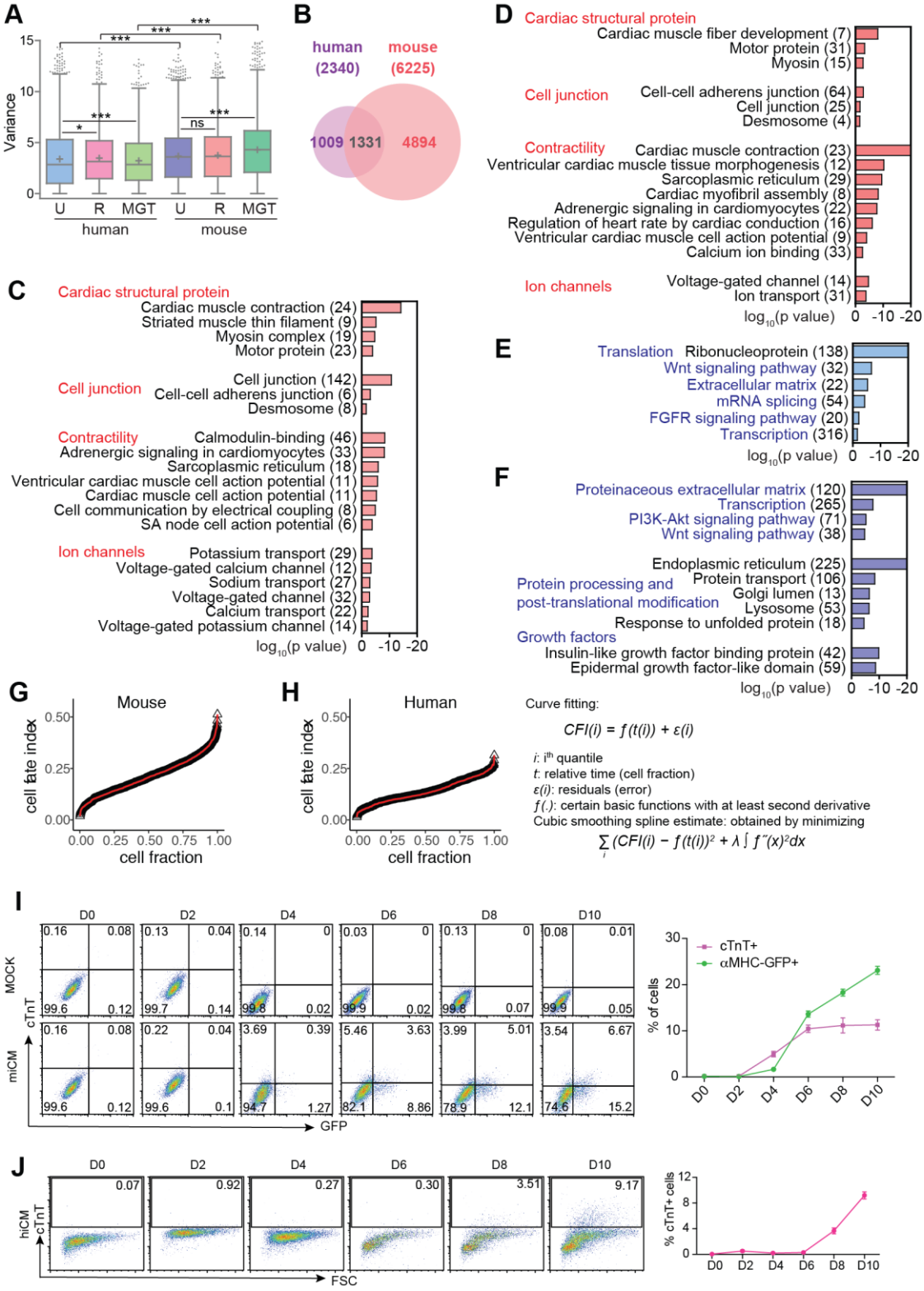


Figure S5. Related to Figures 5. Quantitative assessment of iCM progression in human and mouse cells. (**A**) Box plot showing intercellular transcriptome variance of control and

reprogramming human and mouse cardiac fibroblasts. Central line represents median and + represents mean. *, $p < 0.05$; ***, $p < 0.001$; ns, non-significant. (**B**) Venn plot showing number of genes changed along reprogramming pseudotime during human and mouse iCM reprogramming. (**C-F**) GO analyses of DEG between bulk CF and CM transcriptomes. Representative GO terms were shown for DEGs expressed higher in hCM than hCF (**C**), higher in mCM than mCF (**D**), higher in hCF than hCM (**E**), and higher in mCF than mCM (**F**). Number of genes in each GO term were shown in parentheses. Genes from similar GO terms were combined for the calculation of cell fate indexes and the name of gene lists used for index calculation were color-labeled by red or blue. (**G-H**) Quantiles of CFI over “relative time” (cell fraction) for mouse (**G**) and human (**H**). Curves fitted using cubic smoothing splines were shown in red. Related to Figure 7B, C. Formula is shown on the right. See Methods for more details. (**I)** Representative flow plots and quantification of cTnT+ or αMHC-GFP+ cells during murine cardiac reprogramming. (**J**) Time course flow analysis for cTnT+ cells after infection of hMGT133 in hCF1. Error bars indicate mean ± SEM, N=3.

Figure S6

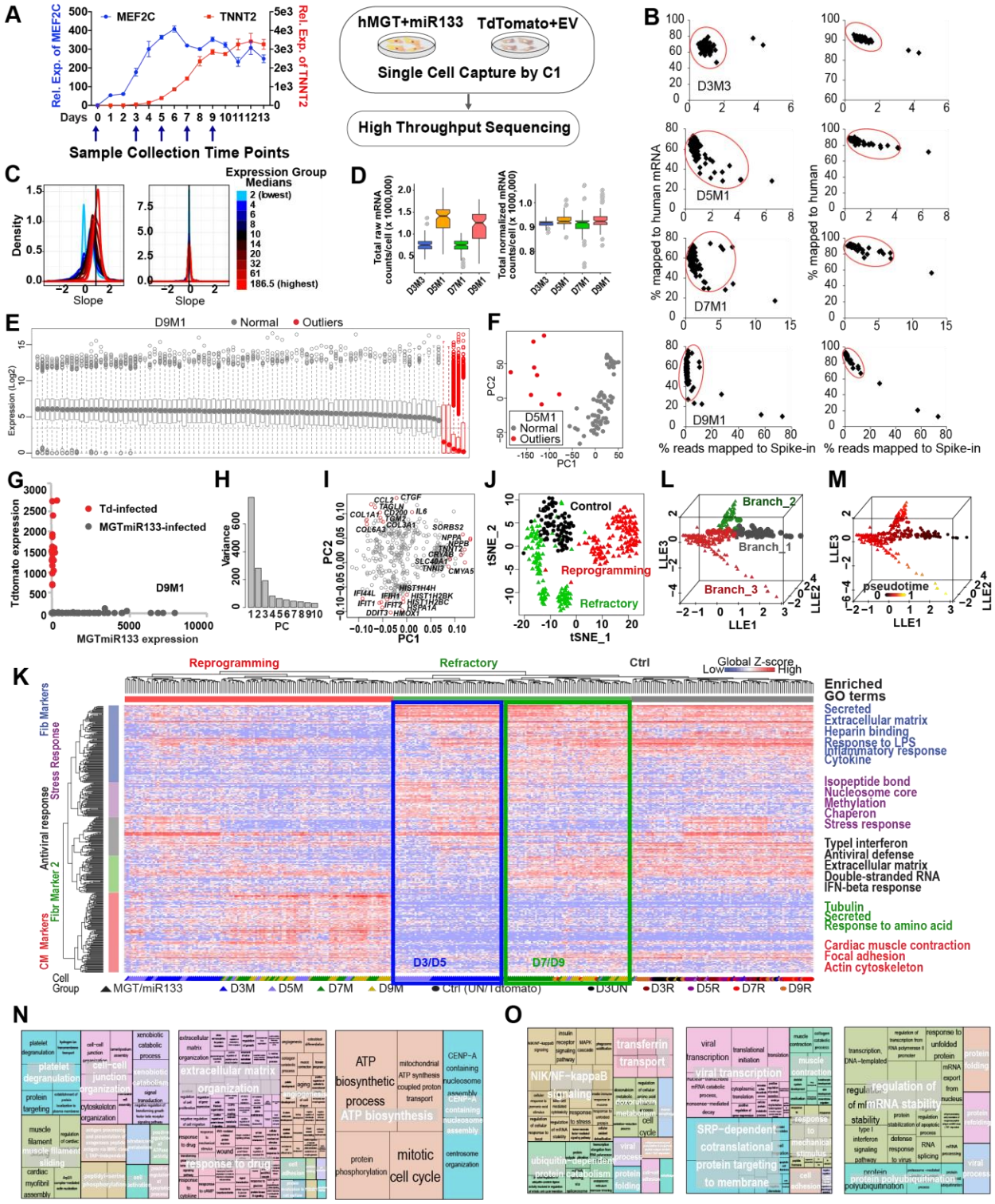


Figure S6. Related to Figure 6. scRNA-seq of additional time points detect cells refractory to iCM reprogramming. (**A**) Time course analysis of MEF2C and TNNT2 expression

determined by RT-qPCR at each day from reprogramming day 0 to day 14. Based on the onset and peak of these two genes, we decided to collect single cell samples as indicated by arrows. The right panel is the simple schematic for time course scRNA-seq. (**B**) Outlier removal. For each of the four single-cell experiments, the percentage of reads mapped to spike-in in each cell was plotted against percentage of reads mapped to human mRNA (left) or human genome (right) in that cell. Cells outside of the red circles are outliers. (**C**) Density distribution of slopes, a measure of count dependence on sequencing depth, of different groups of genes expressed at various levels before (left) and after (right) SCnorm (**D**) Box plots showing total raw (left panel) or normalized (by SCnorm, right panel) human mRNA counts per cell in each single-cell experiment. The central line indicates median. (**E-F**) Removal of additional outliers by median gene expression as shown in box plot in (**E**) or by PCA (**F**). Data from plate D9M1 (**E**) and D5M1 (**F**) were shown as examples here. (**G**) Tdtomato counts were plotted against total counts of M, G, T and miR133 for each plate in order to assign individual cells as “R” (Tdtomato-infected) or MGTmiR133-infected. Plate D9M1 was shown as an example. (**H, I**) PCA scree plot showing variance of top 10 PCs (**H**) and PCA loading plot showing representative genes (**I**). Related to Fig. 6B, C and E. (**J**) Heatmap showing HC results (left) and GO terms enriched in each of the five HC-identified gene clusters (right). Three cell clusters were identified by HC: uninfected or Tdtomato-infected control cells, and MGTmiR133-infected reprogramming or refractory cells. There were 11 cells mis-classified in the control and refractory clusters and their groups were re-assigned for all downstream analyses based on their treatment (Tdtomato or MGTmiR133). (**K**) tSNE plot with cells colored by HC clusters. (**L**) SLICER trajectory colored by its auto-detected branches. (**M**) Pseudotime along the reprogramming and refractory trajectories calculated by selecting the cell on the far right as starting point. (**N, O**) Visualized categorization of full GO terms enriched in each gene clusters along reprogramming (**N**) or refractory (**O**) trajectory.

Figure S7

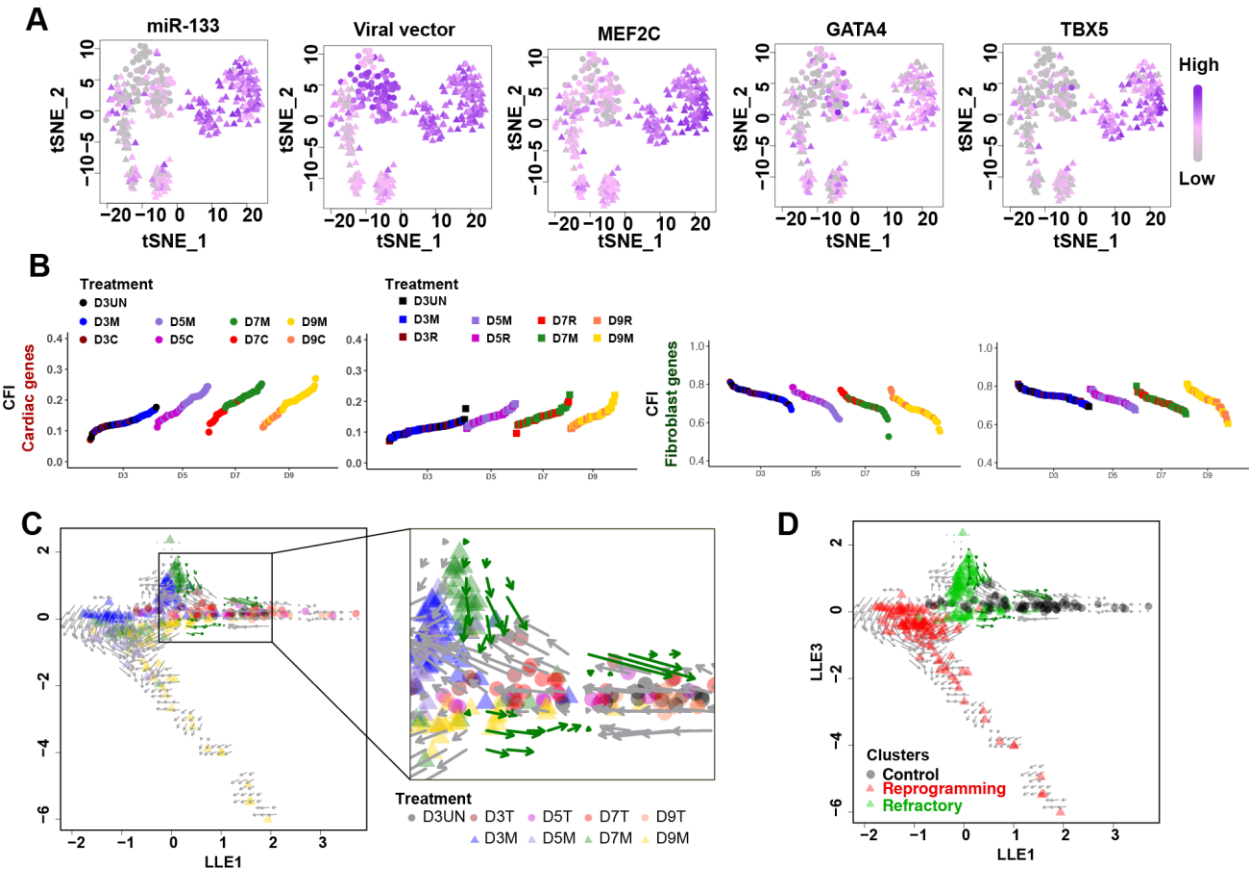


Figure S7. Related to Figure 7. Reprogramming vs. refractory cells. (**A**) Expression level of reprogramming factors mapped to tSNE plot. Purple indicates high expression and gray indicates low. (**B**) Overall cardiomyocyte (left) and fibroblast (right) fate index calculated with cardiac or fibroblast fate- and function-related genes. (**C-D**) RNA velocity analysis showing vector field of RNA velocity projected onto the SLICER trajectory. Arrows indicate the direction and "speed" for the average velocity at a grid of points along the trajectory. Cells colored by treatment (**C**) or HC clusters (**D**).